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16. ABSTRACT

The littoral enclosures research design and protocol were designed to use in developing a field test guidance document to use in the pesticide registration process. With its use, the primary and secondary (ecological) effects of pesticides on the structure and function of lentic ecosystems can be estimated as well as the persistence and fate of pesticides in these environments. In 1986 a study was conducted using chlorpyrifos and in 1989 a study using esfenvalerate was completed. This paper describes the esfenvalerate experiment.

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# Pesticide Testing with Littoral Enclosures.

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## Introduction

The littoral enclosure research design and protocol was developed by researchers from the Duluth Environmental Research Laboratory and the University of Wisconsin-Superior at the request of the U.S. Environmental Protection Agency's Office of Pesticide Programs (OPP) to develop a field testing guidance document to use in the pesticide registration process. With this test protocol it is possible to estimate the primary and secondary (ecological) effects of pesticides on the structure and function of lentic ecosystems, and the persistence and fate of pesticides in these environments. To develop the protocol the first littoral enclosure study was conducted in 1986 with chlorpyrifos. The results are described by Brazner et al. (1988) and Siefert et al. (1989). A second study with esfenvalerate was completed in 1988 (Lozano et al. 1989).

Chlorpyrifos, esfenvalerate, and currently azinphos-methyl, were chosen for study because of the high priority status given them by OPP. Chlorpyrifos was used in the initial littoral enclosure study because Environmental Research Laboratory-Duluth researchers had previous experience testing this pesticide both in the laboratory and in outdoor systems and numerous laboratory and field study results that used chlorpyrifos as a test chemical were available. With esfenvalerate, the limited information on the effects of pyrethroids on natural aquatic systems and the trend towards widespread use of synthetic pyrethroids was considered. This paper describes only the esfenvalerate study.

The littoral enclosure design was developed to have a wide range of applicability to allow measurement of changes in ecologically important attributes in response to pesticide additions, and to meet the field testing requirements of OPP. To accomplish this, it was essential that the system: (a) be suitable for use in natural or artificial ponds or lakes, (b) be relatively simple and economical, (c) be conducive to accurate pesticide chemistry studies, (d) allow for true experimental replication, (e) include the natural undisturbed sediment and shoreline, (f) be representative of the

aquatic ecosystem being partitioned, (g) include habitats impacted directly or indirectly by actual-use, (h) maintain its structural integrity and bottom seal for at least 1 full field season, and (i) be large enough to allow the removal of samples necessary to characterize the parameters of interest without serious disruption to the enclosed ecosystem. Littoral enclosures meet all of these requirements.

## Littoral Enclosure Description

Eighteen enclosures (5 m x 10 m) were constructed on a 2 ha pond near Duluth, Minnesota. The pond contains a diverse community of aquatic plants and animals. The littoral areas are well-developed with cattails, pond grasses and aquatic macrophytes growing in unconsolidated, highly organic sediments. Each enclosure consisted of 5 m of natural shoreline and three walls made from an inert polyolefin plastic, Scrimweve$^{R}$ (#888 H UV Clear Scrimweve$^{R}$, StoCote Products Inc., Richmond, IL) built to enclose an area of 0.004 ha (0.01 acre). The average maximum depth of the enclosures on June 1, 1988 was 1.1 m. Since the enclosures included natural shoreline, littoral zone and undisturbed sediments, all components of the habitat and food supply for endemic pond organisms were available. This allowed replication to be incorporated into the experimental design of this field ecosystem study without seriously compromising the ecological integrity of the results.

## Experimental Design

Responses of six major trophic levels and the physical and chemical environment to two applications of esfenvalerate were studied during the spring and summer of 1988 (Table 1). Esfenvalerate was applied as Asana$^{R}$ 1.9 EC (228 µg/l active ingredient) on June 20 and July 18, 1988 between 8:30 a.m. and 2:30 p.m. (EDT). The 18 littoral enclosures were treated with a surface spray using a hand sprayer with a 2.4m wand to obtain 5 nominal treatment concentrations (0.01, 0.08, 0.2, 1.0 and 5.0 µg/l) and a control in each block of six enclosures.

Table 1. Samples collected from littoral enclosures from May 31, 1988 to September 20, 1988, tabulated by type and expected endpoint.

| Type | Endpoints |
|---|---|
| Water Chemistry | pH, Specific conductance, Turbidity, Apparent color, Alkalinity, Dissolved organic carbon, Calcium, Magnesium, Sodium, Potassium, Hardness, Total dissolved phosphate, Ortho-phosphate, Dissolved oxygen, Esfenvalerate concentration |
| Sediments | Esfenvalerate concentration, Organic carbon, Grain size |
| Enclosure Wall Material | Esfenvalerate concentration |
| Phytoplankton | Species composition, Biomass, Chlorophyll *a* |
| Periphyton | Species composition, Biomass, Chlorophyll *a* |
| Macrophytes | Species composition, Biomass, Esfenvalerate concentration |
| Microinvertebrates | Toxicity, Species composition and abundance |
| Macroinvertebrates | Toxicity, Species composition and abundance |
| Fish | Toxicity, Diet and growth, Species composition, Reproductive success, Esfenvalerate concentration |

The concentration range was based on several criteria. These included 1) encompassing a wide range of the laboratory derived $LC_{50}$ values for a variety of aquatic organisms and the responses of Mixed Flask Cultures (MFC) microcosms, 2) the need to have a no-effect concentration, 3) simulating a "worst case" aquatic concentration, 4) use of effects data from previous field studies done by the National Fish Contaminant Research Center (USFWS, Columbia, MO; pers. comm., T.W. LaPoint) and 5) to be similar to those used in concurrent studies with the same compound in other parts of the United States.

Since this study was intended to evaluate a general testing protocol, loading from runoff was not used as a primary criterion to select the concentration ranges. Runoff estimates are highly variable and depend upon factors such as pesticide formulation, rainfall, soil type, extent of buffer zone, and size of the watershed. Also, no report of measured esfenvalerate concentrations from agricultural runoff in natural waters was found in the literature. However, assuming a "typical" situation of average slope, soil type, rainfall, and application rate, and based on the runoff estimates of Wauchope (1978) for the majority of commercial pesticides, a total of 0.5% may be lost to runoff. Therefore, a typical Asana$^R$ application rate for control of the Colorado Potato Beetle of 0.028 kg/ha would be expected to produce an aquatic loading rate of 0.00014 kg/ha which is within the selected loading range used in these experiments (0.00003 - 0.028 kg/ha).

The application method and frequency were selected based on the regional use pattern of Asana$^R$. The major use is on potatoes to control the Colorado Potato Beetle and is applied by air or ground spray, 2-3 times per growing season. In this study the pesticide was applied twice to the surface of each enclosure to simulate drift and reflect application frequency on agricultural crops in the Upper Midwest.

Due to varying shoreline topography the enclosures were built in 3 blocks, each containing 6 enclosures; block locations were selected to maximize habitat similarity within and between blocks. The west and middle blocks of enclosures were separated by approximately 30 m of shoreline, and

the middle and east blocks by approximately 100 m. Random assignment of enclosures to treatment concentrations within each block is shown in Figure 1. Due to the paucity of laboratory and field studies with this compound, its high toxicity, and the relatively high uncertainty associated with the Estimated Environmental Concentration (EEC) under normal use patterns, it was decided that a complete randomized block design was best suited for testing the effects of esfenvalerate in the littoral enclosures and a regression approach was the most suitable analytical technique for delineating effects.

## Environmental and Pesticide Chemistry Studies

Physical and chemical characteristics of all enclosures and the open pond were monitored both before and during the experiments. Nutrient analyses for total dissolved phosphorus, orthophosphorus, cations (Ca, Mg, Na, K), and dissolved organic carbon provided concurrent biological studies with nutrient data and characterization of the test water. Climatological data were obtained from the National Weather Service Weather Station which is located within 1 km of the study site.

The physical dimensions including length, width, mean depth, and volume were measured for all enclosures after the barriers were in place. The volume of each enclosure was used to calculate the pesticide application rate and was adjusted at the time of each application to account for normal water level fluctuations.

Esfenvalerate chemistry studies included characterizing the distribution within enclosures. This included the determination of residues in the water, enclosure wall material, macrophytes, and sediment. Also, the uptake of esfenvalerate by the fathead minnow, Pimephales promelas was studied.

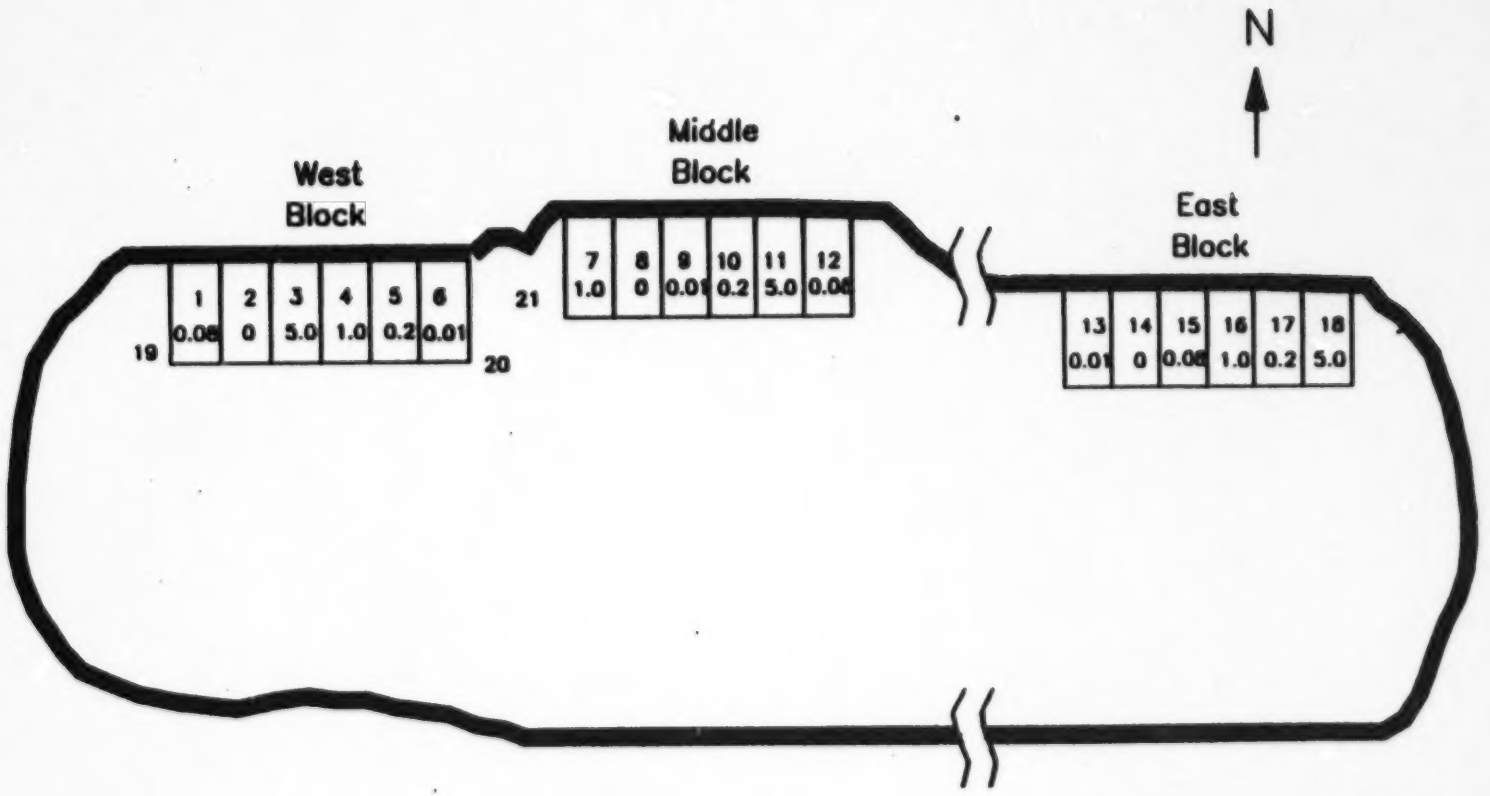


Figure 1. Diagrammatic representation of pond study site, 1988 littoral enclosure study. Enclosures include enclosure number and esfenvalerate concentration (ug/l). Open pond sites denoted by 19, 20, and 21.

## Biology Studies

The biological community of the littoral zone constitutes an important, dynamic and complex part of the pond ecosystem. The objectives of the biological studies conducted within littoral enclosures were to measure the direct and indirect effects esfenvalerate on indigenous organisms and to estimate the impact on the whole littoral community. Laboratory single species bioassays were conducted to determine the relative sensitivities of test pond populations and to estimate pesticide concentrations which would result in different levels of change in the littoral enclosures. The observed lethal concentration (LC) and the effect concentration (EC) were used to estimate those concentrations that would cause change. In addition, MFC microcosm experiments were conducted in the laboratory to explain how esfenvalerate could affect community structure and system level processes such as primary productivity and respiration. The lowest observable effect concentration (LOEC) and the most sensitive ecosystem components were identified. The bioassays were also used to evaluate the accuracy of the laboratory-to-field predictions.

The plant and invertebrate studies in the littoral enclosures were used to describe the changes in species composition and population abundance over the test period, both in the enclosures and in the open pond littoral zone, and to quantify the effect of esfenvalerate on the littoral communities as a whole. Multivariate statistical techniques were used to assess the response of the whole invertebrate community to the pesticide.

Four fish studies were conducted in the littoral enclosures. They included:

1) a juvenile bluegill survival, growth and diet study.
2) a larval cyprinid survival, growth and diet study.
3) an endemic fish trapping study.
4) a bluegill reproductive success study.

The purpose of the juvenile bluegill study was to determine the effect of multiple applications of esfenvalerate on the survival, growth and diet of

juvenile bluegill sunfish, Lepomis macrochirus. Both primary and secondary effects of the pesticide application were investigated. Based on limited laboratory and field toxicity data, bluegills were expected to be quite sensitive to this insecticide.

The purpose of the larval cyprinid study, while similar to that of the juvenile bluegill study, allowed the determination of the effects of esfenvalerate on a different life stage. In the early life stages (larval/post-larval) fish are often most sensitive to toxicants (McKim 1977). Because of its abundance in the pond, the larval stage of northern redbelly dace (Phoxinus eos Cope) was selected as the primary test species for this study.

The endemic fish trapping study was conducted to determine the number of endemic fish species present in the enclosures, to provide an estimate of their density and biomass in each enclosure, and to reduce population numbers before pesticide application so as to assure minimal impact on invertebrate forage populations.

The objectives of the bluegill reproductive success study were to determine the effects of esfenvalerate on adult survival, spawning behavior, embryo hatchability, larvae survival and growth, and young-of-the-year biomass. An accurate measure of these biological endpoints would allow the prediction of pesticide effects on the well-being of populations of this important sport fish.

## Results

This esfenvalerate study demonstrates that the littoral enclosure system and protocol is very useful in investigating the effects of pesticides in lentic ecosystems. The results provide insight into the likely effects caused by the addition of esfenvalerate to an aquatic ecosystem.

The esfenvalerate water chemistry studies revealed that the deviation of the maximum esfenvalerate concentration from the EEC was 4.6% above following the first application, and 11.0% below after the second application. The

maximum concentration in the water column occurred 1 to 3 h after application, and no esfenvalerate was detected in the water 48 h after application at any treatment concentration. In addition, the half-life of esfenvalerate in the water column was inversely proportional to the initial treatment concentration. The half-life ranged from 8 h in the 5.0 µg/l treatment enclosures to 24 h in the 0.08 µg/l treatment enclosures. A concentration gradient was also observed in the water column, revealing that little esfenvalerate penetrated to depths greater than 50 cm and that maximum concentrations occurred in the top 27 cm of the water column. Since this top 27 cm represents 38 to 52% of the water volume in the enclosures, a large percentage of the enclosed biota was exposed to maximum esfenvalerate concentrations.

The distribution of esfenvalerate within the enclosures was characterized by two phases: 1) a water phase during the first 4 days after application, where the majority of the pesticide was in the water column, and later, 2) a sediment phase where the sediment contained an increasing percentage of the esfenvalerate. Macrophytes, enclosure walls, and fish were also found to contain small but significant amounts of the pesticide during the first two to three weeks after application. The maximum amount of esfenvalerate accounted for in a mass balance completed after either application was 35%, found 1 day after the first application, when 128 mg was applied. However, there were several indications of cumulative sorption of esfenvalerate, in that significantly greater sorption to the enclosure wall and sediment was observed after the second application as compared to the first.

This persistence in the wall and sediments probably played a role in sustaining some of the longer lasting biological effects that were observed. Many invertebrate populations were significantly reduced at esfenvalerate concentrations greater than or equal to 0.08 µg/l, and the effects were often apparent at the end of the study. Esfenvalerate exposures of 1.0 and 5.0 µg/l

resulted in large reductions or elimination of some copepod and cladoceran taxa. Cladocerans, copepods, amphipods, and chironomids were among the most sensitive taxa. The no-effect concentration for the majority of invertebrates was 0.01 µg/l, although there appeared to be some subtle, short-lived effects on a few populations at this treatment level. One cladoceran (Ilyocriptis), the ostracod, Cyclocypris, several genera of rotifers, oligochaetes and snails all increased in abundance after the pesticide application. These increases were probably indirect effects of treatment that resulted from direct effects of the pesticide on some of the above species, predators, competitors, and/or food sources.

The changes observed in the invertebrate community structure could profoundly affect other components of the food chain. The loss or reduction in many of the invertebrate populations appeared to result in changes in the diets, growth, and possibly, survival of fish in the enclosures.

The 0.01 µg/l esfenvalerate concentration did not significantly alter any of the fish parameters studied. At concentrations of 0.08 µg/l, juvenile bluegills exhibited slower growth and lower total biomass than the control fish. In the 0.20 µg/l treatment enclosures, the diets and growth rates of both larval cyprinids and juvenile bluegills were affected. Long-lasting (up to 53 days) changes in diets were observed for both species, and growth rates of juvenile bluegill were reduced by nearly 50% compared to the controls. The 1.0 and 5.0 µg/l concentrations were extremely toxic to all life stages of all fish studied. No fish survived the 5.0 µg/l concentration. No juvenile bluegills and only 55% of the larval cyprinids survived the 1.0 µg/l concentration. In addition, the 1.0 µg/l treatment reduced total fish biomass, delayed spawning of adult bluegills, caused mortality of larval bluegills prior to swim-up and slowed growth of surviving bluegill larvae.

There were no direct or indirect effects of the esfenvalerate applications on the algae and macrophyte communities as estimated from chlorophyll a and biomass measurements. Generally, the peak abundance of

phytoplankton occurred in mid-summer, and periphyton and macrophyte biomass gradually increased throughout the summer in all enclosures.

The littoral enclosure research design presented here is an excellent experimental system that can estimate the persistence and distribution of a pesticide allowed to enter an aquatic ecosystem, estimate the likely primary and secondary (ecological) effects on biota, and allow comparisons of laboratory toxicity data to actual effects in a controlled, yet natural, aquatic system.

## References


Brazner, J.C., S.J. Lozano, M.L. Knuth, S.L. Bertelsen, L.J. Heinis, D.A. Jensen, E.R. Kline, S.L. O'Halloran, K.W. Sargent, D.K. Tanner and R.E. Siefert. 1988. The effects of chlorpyrifos on a natural aquatic system: A research design for littoral enclosure studies and final research report. U.S. EPA, ERL-Duluth, MN.

Lozano, S.J., J.C. Brazner, M.L. Knuth, L.J. Heinis, K.W. Sargent, D.K. Tanner, L.E. Anderson, S.L. O'Halloran, S.L. Bertelsen, D.A. Jensen, E.R. Kline, M.D. Balcer, F.S. Stay and R.E. Siefert. 1989. Effects, Persistence and Distribution of Esfenvalerate in Littoral Enclosures. U.S. EPA, ERL-Duluth, MN.

McKim, J.M. 1977. Evaluation of tests with early life stages of fish for predicting long-term toxicity. J. Fish. Res. Board Can. 34:1148-1154.

Siefert, R.E., S.J. Lozano, J.C. Brazner and M.L. Knuth. 1989. Littoral enclosures for aquatic field testing of pesticides: The effects of chlopyrifos on a natural system. Misc. Publ. Series, Entomological Society of America (in press).

Wauchope, R.D. 1978. The pesticide content of surface water draining from agricultural fields: A review. J. Environ. Qual. 7(4):459-471.